FISHERY RESEARCH

GENETIC DIVERSITY AND DIFFERENTIATION OF SHOSHONE SCULPIN *COTTUS GREENEI*

COMPLETION REPORT
January 1, 2010 – June 30, 2011

Prepared by:

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IDFG Report Number 11-20
November 2011

Genetic Diversity and Differentiation of Shoshone Sculpin *Cottus Greenei*

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By

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

INTRODUCTION

The Shoshone sculpin *Cottus greenei* is one of eight sculpin species found in Idaho (Simpson and Wallace 1982). Although some of Idaho's sculpin species are widely distributed throughout the state, Shoshone sculpin are endemic only to springs and tributaries of the Snake River within the confines of the Hagerman Valley (Griffith and Kuda 1994). Because of its limited distribution and perceived possible impacts to remaining habitat (Griffith and Kuda 1994), Shoshone sculpin are recognized as a species of special concern by the Idaho Department of Fish and Game (IDFG), and a species of concern by the USFWS (IDCDC 2003). Recently, IDFG and Idaho Power biologists have collaborated on research projects aimed at better understanding the distribution, life history, and genetic population structure of the species. This study's goal is to provide genetic information on the genetic diversity, structure, and effective population size of populations across the species' present range. This document reports on the second year of this project.

MEASURABLE OBJECTIVES (YEAR 2)

- To genotype approximately 560 samples from ~10-14 populations with ~7 microsatellite DNA loci.
- To prepare a final report for the 2010-2011 agreement period by June 30, 2011.

METHODS

Population Sampling

Shoshone sculpin were sampled (fin tissue) from ten sites in the fall of 2009 and the spring of 2010 using either electroshocking techniques or minnow traps (see cover photo). These sites and ten additional sites collected in 2008 are shown in Table 1 and Figure 1. Technicians were provided photographs and diagnostic phenotypic characteristics to differentiate mottled sculpin *C. bairdii* from Shoshone sculpin at sample sites. Ten fish from each site were kept following fin tissue sampling to serve as voucher specimens and were sent to the Orma J. Smith Museum of Natural History in Caldwell, Idaho (Donald W. Zaroban, Curator of Fishes) for archiving. These whole samples, as well as all fin tissue samples, were stored in 100% non-denatured ethanol.

DNA Extraction and Microsatellite Screening

DNA was extracted from fin tissue using Nexttec extraction kits (Nexttec, Leverkusen, Germany) following the manufacturer's instructions (fish tissue protocol; version 4.0). In the first year of this study, we were successful in identifying 18 microsatellite loci that amplify well and exhibit variation within and between Shoshone sculpin populations (Campbell et al. 2010). For the second year of this study, we optimized 12 of the 18 loci, ones exhibiting the highest diversity, into two PCR panels. The 12 loci were *Cba42, Cott100, Cott105, Cott113, Cott130, Cott207, CottES10, Cba310, Cgo114, Cgo33, Cott118, and LCE89*. Most of these loci used in this study either do not amplify in mottled sculpin or exhibit allele sizes that are diagnostic between the two species, allowing us to check phenotypic identifications (Campbell et al. 2010). Information on primer sequences for these loci is available in Campbell et al. (2010). Information on PCR reactions and thermal cycling conditions for the two specific multiplex panels used in

this study is available from the author upon request. Resulting amplification products for each panel were sized by capillary electrophoresis on an automated ABI 3100 using the molecular standard GeneScan™ 500 LIZ® and GeneMapper® 3.5.1 software (Applied Biosystems).

Statistical Analyses

Samples from year one (477 samples; 10 collection sites) and from year two (834 samples; 10 collection sites) were combined for all analyses and reporting. Data generated for each population was tested for Hardy–Weinberg equilibrium and linkage disequilibrium with GENEPOP on the Web (Raymond and Rousset 1995). An alpha value of 0.05 was chosen for statistical significance, but was adjusted for multiple tests using Bonferroni's correction (Rice 1989). Genetic diversity was measured by the number of alleles per locus (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) using the Microsatellite Toolkit for Microsoft Excel™ (Park 2001).

GENEPOP on the Web was used to perform exact tests to assess the significance of allelic differentiation between pairs of populations and to estimate pairwise population differentiation (F_{ST}; Weir and Cockerham 1984). To examine genetic relationships among populations, genetic distances (Cavalli-Sforza and Edwards 1967) between all populations were estimated in GENDIST in PHYLIP v. 3.5 (Felsenstein 1993). A neighbor-joining dendrogram was generated from these genetic chord distances with the program FITCH in PHYLIP. Bootstrap replicates of 1000 iterations were attained with SEQBOOT and a consensus tree was formed with CONSENSE in PHYLIP. The dendrogram generated in PHYLIP was plotted as a radial tree using TREEVIEW (version 1.6.6, Page 1996).

To test whether genetic differentiation between collection sites was associated with geographic distance, a Mantel's test (Mantel 1967) was performed from the comparison of population pairwise FST/(1-FST) values against population pairwise straight line geographical distances (Ln) using the program ISOLDE in GENEPOP.

Contemporary effective population size, N_E, was estimated with the linkage disequilibrium method of Waples (2006) using the software program LDNE (Waples and Do 2008). Alleles with a frequency <0.02 were excluded to decrease bias (Waples 2006) and confidence intervals were estimated with the jackknife method. Sample sizes for most collection sites averaged ~50. However, over 340 samples were collected from Fisher Lake and 315 were genotyped. To test the influence of sample size on N_E estimates (England et al. 2006), we ran LDNE with sample sizes from Fisher Lake of 50, 100, 150, 200, 250, and 300.

Regarding estimates of contemporary N_E; these can be made from a single year sample (e.g., linkage-disequilibrium method), but are based on several assumptions including that samples are drawn from one breeding generation (Waples 2006). In situations where samples are drawn from a population with overlapping generations but cohorts can be identified, it is still possible to provide an estimate of N_B (the effective number of breeders that produced the sample) (Waples 2006). An attempt was made to age Shoshone sculpin from several sites using otoliths. However, clear annual growth increment patterns were not present in the samples examined (Liz Mamer, IDFG, personal communication). In this study, estimates of effective size were still calculated using LD procedures from samples of adults that were likely of mixed ages. However, the effects of age structure have not been evaluated for any single-sample N_E estimator (Robin Waples, NOAA, personal communication), and it was recognized that the resulting values would likely be estimating something intermediate between N_B and N_E (Waples 2005) and might be imprecise and difficult to interpret.

To assess whether populations showed evidence of undergoing a recent bottleneck or expansion event, we tested for heterozygote excess or deficiency, respectively, using the software program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999). The significance of the test was assessed using Sign, Wilcoxon, and L-shape tests under the stepwise mutation (SMM) and two-phase mutation models (TPM) suggested for microsatellite evolution.

RESULTS

Tests for Hardy–Weinberg Equilibrium and Linkage Disequilibrium

A total of 1311 Shoshone sculpin samples were included in analyses. Of 240 (20 collection sites X 12 loci) tests for deviations from Hardy–Weinberg equilibrium, 10 were significant at $\alpha = 0.05$, but this was not higher than expected by chance (240 X 0.05 = 12 expected from type I error of 0.05) and no collection sites or loci consistently deviated from Hardy–Weinberg equilibrium. No HWE tests were significant following Bonferroni correction (0.05/240 = 0.0002). Of the 1320 tests for linkage disequilibrium (12 loci X 12 = 144 – 12 = 132/2 = 66 X 20 collection sites = 1320), 82 were significant at $\alpha = 0.05$, which was slightly higher than expected by chance (1320 X 0.05 = 66 expected from type I error of 0.05). However, no more than four tests clustered around a particular locus pair, and only two tests were significant following Bonferroni correction (0.05/1320 = 0.00004), indicating that none of these loci were closely linked.

We observed seven samples with genotypes indicative of mottled sculpin. All were from Briggs Creek. These samples were removed from further analyses. No samples exhibited genotypes with both mottled sculpin and Shoshone sculpin alleles, indicative of hybrids.

Across the 20 populations examined, the total number of alleles per locus observed ranged from seven alleles at *Cott105 and Cott118* to 25 alleles at *Cott207*. Populations exhibited large variation in genetic diversity among sites (Table 1). Nine sites exhibited heterozygosity estimates lower than 40% (average 33.9%; range 21.9% to 39.2%). Allelic variation in these populations averaged 3.5 (range 2.4 to 4.9). The remaining 11 sites exhibited heterozygosity estimates greater than 45% (average 56.3%; range 45.7% to 62.1%). Allelic variation in these populations averaged 5.8 (range 4.3 to 7.2).

Genetic Differentiation and Structure

The level of genetic differentiation, as measured by F_{ST} estimates, ranged from <0.001 (eight pairwise comparisons) to 0.62 for Pottery House Springs and Briggs Springs (Table 2). The distance between Pottery House Springs (third farthest downstream location) and Briggs Spring (farthest upstream location) is ~45 km. The largest distance between sites that exhibited an F_{ST} <0.001 was ~3.5 km (Riley Creek and Sand Springs). All but two population pairwise exact tests (lower Riley Creek versus Sand Springs and Sculpin Springs) were highly significant and the average pairwise F_{ST} across all sites was 0.24, indicating significant genetic differentiation among most sites.

The neighbor-joining dendrogram indicated that genetic population structuring was generally correlated to geography (Figure 2). Populations (#1-3, 6 and 7) from creeks and springs entering the Snake River north of Hagerman, Idaho, clustered together with 100%

bootstrap support. Populations (#9 and #11-20) from creeks and springs entering the Snake River south of Hagerman (upstream) clustered together with 100% bootstrap support. The exceptions to this pattern were lower White Sand Springs (#5) and the Malad River (#4), which did not cluster with any populations, and two isolated populations on upper Riley Creek (#9) and upper Bickel Springs (#11), which are located south of Hagerman but cluster with downstream collection sites.

Finer-scale structure among geographically proximate sites was also observed (Figure 2). Starting downstream (site #1) and moving upstream; samples from Montana Mining Ditch, Sullivan Springs, and Pottery House Springs (#1, 2, and 3) clustered together with 99% bootstrap support. Samples from Billingsley Creek (#6) and Fisher Lake (#7), both in the Billingsley Creek drainage, clustered together with 99% bootstrap support. Samples from lower Riley Creek (#9), lower Bickel Springs (#11), Thousand Springs (#12), Sculpin Springs (#13), and Sand Springs (#14) clustered together with 72% bootstrap support. Finally, samples from Blue Hearts Spring (#15), lower Box Canyon (#16), upper Box Canyon (#17), Blind Canyon (#18), Banbury Springs (#19), and Briggs Creek (#20) all clustered together with 93% bootstrap support.

Isolation by Distance, Effective Population Size and Bottlenecks

A significant pattern of isolation by distance was observed from the comparison of genetic and geographic distance for the 20 study populations (Figure 3; $R^2 = 0.27$, P-value <0.0001).

Effective population size estimates using LDNE were highly variable among sites (Table 2). Of the positive point estimates observed, Billingsley Creek (#6) had the lowest N_E estimate (114.5) and lower Bickel Springs had the highest (19674.3). Corresponding confidence intervals for all but one population included infinity. Five sites yielded negative point estimates. The test of adjusting sample sizes (50-300) for the Fisher Lake population also yielded large variations in N_E estimates (Table 3). The smallest estimate of N_E was observed with a sample size of 50 (149.8) and the largest was observed with a sample size of 150 (4119.4). The sample size of 200 yielded a negative point estimate and sample sizes of 250 and 300 yielded estimates of 1729.6 and 2156.2, respectively. Corresponding confidence intervals for all six samples sizes included infinity.

No populations showed evidence of a recent bottleneck under any of the three tests for both mutational models (Table 4). A general pattern of heterozygosity deficiency was observed for all sites, and eight sites exhibited significant p-values (<0.0025, Bonferroni correction: [0.05/20 = 0.0025]) under the Wilcoxon test of heterozygosity deficiency, which is considered to be the most powerful of the three tests when less than 20 loci are used (Piry et al. 1999).

DISCUSSION

The genetic population structure of a species refers to the amount and distribution of genetic variation within and between populations. This structuring has specific implications for conservation and management efforts. Results from this study clearly show that Shoshone sculpin are highly structured, with substantial genetic differentiation observed between most populations. This structuring is likely a product of a number of different influences. Freshwater sculpin generally are sedentary, with low rates of dispersal and relatively small home ranges (Hendricks 1997; Hudy and Shiflet 2009). The evidence of isolation by distance across the

range of Shoshone sculpin is a pattern compatible with limited gene flow and random genetic drift within populations. Shoshone sculpin are also habitat specialists, endemic to the springs and spring creek habitats along the Thousand Springs Formation. These springs are naturally fragmented and have been extensively developed as part of hydroelectric facilities, irrigation, and fish culture operations (Griffith and Kuda 1994). These localized anthropogenic influences along with decreases in spring discharges (naturally and anthropogenically influenced), have likely further fragmented populations and reduced available habitat (Griffith and Kuda 1994). These types of influences can impact population size and the amount of gene flow among adjacent populations, which in turn can impact genetic diversity and differentiation of populations. Genetic diversity was highly variable among sites, and populations that are known to be geographically isolated due to manmade barriers in the forms of dams, weirs, or diversions (e.g., Briggs Creek [#20], Banbury Lake [#19], Fisher Lake [#7]), generally exhibited lower levels of genetic variation and higher levels of divergence from other populations. Alternatively, there were examples of geographically proximate, physically connected populations, which exhibited higher levels of genetic diversity and lower levels of genetic differentiation (lower Riley Creek [#9], lower Bickel Springs [#11], Thousand Springs [#12], Sculpin Springs [#13] and Sand Springs [#14]).

It was expected that these patterns might be reflected in estimates of effective sizes of these populations. Effective population size is an important parameter to estimate because it is a measure of the number of individuals in a population that contribute offspring to the next generation and their relative contribution. Effective population size is almost always smaller than census size (which biologists have traditionally attempted to measure) and summarizes the magnitude of genetic drift and increase in inbreeding occurring in a population (Wright 1931). However, estimates of Shoshone sculpin N_E were imprecise, as evidenced by negative point estimates and confidence intervals which all included infinity.

There are a number of confounding variables that may have contributed to the low precision in N_E estimates including violations of assumptions associated with closed populations and overlapping generations, the number of loci used and allelic diversity, as well as sample size. Although we picked the 12 loci exhibiting the highest level of variation across study populations, allelic variation was low. For each pair of loci, linkage disequilibrium is computed for each of the allelic combinations and an overall mean is calculated for that pair. The total number of independent comparisons across all pairs of loci provides a measure of precision associated with the overall mean (Waples and Do 2008). With regards to sample size, it has been shown via modeling that when the effective population size is substantially greater than the sample size, the original LD estimator was strongly biased downward (England et al. 2006). Although the corrected LD methods used in LDNE reduce bias, precision is still quite low when true N_E is large (Waples 2006). In addition, all methods of estimating N_E have difficulty obtaining reliable estimates for large populations and have low power in distinguishing a large N_E from infinity (Waples and Do 2010; Luikart et al. 1999).

For the Fisher Lake population, we had an opportunity to run LDNE with a series of subsamples of increasing size. It has been suggested that when doing this type of subsampling test that an inflexion point should be observed when the sample size exceeds the true N_E (England et al. 2006). We did not observe a clear inflexion point with sample sizes up to 300, which may suggest that the true N_E is being underestimated by an unknown amount (Waples 2006). A previous study of mottled sculpin suggested that the total number of effective breeders was an order of magnitude smaller than the total number of potential breeding pairs (Fiumera et al. 2002). This is consistent with the observation that the N_E for many species is an order of magnitude less than the number of individuals censused (Moritz and Sherwin 2009). Based on

mark-recapture efforts that were conducted during genetic sampling, the Fisher Lake and Banbury Lake adult populations were estimated to be ~15,000 and ~20,000, respectively, (IDFG and IPC unpublished data), and we might expect that the effective sizes of these populations could be quite high (~1,500–2,000).

Finally, despite natural and anthropogenic fragmentation, losses in available habitat and highly variable levels of genetic variation (with some sites exhibiting more than half the diversity of other sites), no populations showed evidence of recent bottlenecks. Instead, we found evidence for population expansion, which can eliminate evidence of past bottlenecks.

Several important accomplishments were made during the two years of this project. The project was successful in identifying a suite of microsatellite loci that amplify well and exhibit variation within and between Shoshone sculpin populations. Many of these loci also differentiate *C. greenei* and *C. bairdii* allowing assessments of hybridization between these sympatric species. The project provides the first assessment of genetic diversity and structure across the species range and confirms that Shoshone sculpin are a highly structured species indicating that it will be important that future conservation efforts are focused at the population level.

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Table 1. Population, sample size, expected heterozygosity (H_E), number of alleles per locus (N_A), and effective population size estimates from LDNE (with 95% CI) of 10 Shoshone sculpin collection sites sampled in 2009 and 2010. Samples from 10 collection sites sampled in 2008 (from the first year of the project) are also shown for comparison purposes.

Population	Collection Site #	IDFG Access #	Year	N	H_E	H_O	N_A	N_E	$N_E^{(95\% L)}$	$N_E^{(95\%U)}$
Montana Mining Ditch	1	2445-2685	2009	35	0.38	0.37	3.1	-131.8	68.2	∞
Decker/Sullivan	2	2446-2686	2009	70	0.37	0.38	3.4	-6256.8	95	∞
Unm. Pottery House	3	2447-2687	2009	53	0.35	0.34	3.1	441.1	61.3	∞
Malad River	4	2240-2471	2008	49	0.50	0.49	4.9	-1972.7	128.4	∞
Lower White Springs	5	2444-2684	2009	80	0.48	0.49	4.9	937.5	126.5	∞
Billingsley Creek	6	2241-2472	2008	50	0.33	0.32	2.7	114.5	34.1	∞
Fisher Lake	7	CgrBLGY10C*	2010	50*	0.36	0.36	4.9	149.8	37	∞
Riley Creek (upper)	8	2451-2691	2009	57	0.39	0.38	4.1	687.9	100.1	∞
Riley Creek (lower)	9	2452-2692	2009	54	0.62	0.61	6.7	271.2	82.1	∞
Bickel Springs (upper)	10	2358-2604	2008	50	0.36	0.35	3.3	131.9	32.6	∞
Bickel Springs (lower)	11	2450-2690	2009	50	0.61	0.59	6.4	19674.3	224.5	∞
Thousand Springs	12	2236-2467	2008	50	0.62	0.59	7.2	132.4	69.2	605.3
Sculpin Springs	13	2239-2470	2008	50	0.62	0.62	6.7	175	77.2	∞
Sand Springs	14	2237-2468	2008	50	0.62	0.59	7.0	-1169.3	226.1	∞
Blue Hearts Springs	15	2259-2490	2008	23	0.55	0.59	4.4	270.4	43.5	∞
Box Canyon (lower)	16	2238-2469	2008	50	0.57	0.56	6.1	131.1	50.8	∞
Box Canyon (upper)	17	2235-2466	2008	49	0.46	0.43	4.3	150.8	56.4	∞
Blind Canyon	18	2449-2689	2009	55	0.56	0.55	5.8	1485.7	106	∞
Banbury Springs	19	2186-2414	2008	56	0.33	0.32	4.3	186.2	48.3	∞
Briggs Creek	20	2448-2688	2009	65	0.22	0.22	2.4	-230.3	83.2	∞

*This collection was inventoried in new Progeny database.

Table 2. Pairwise F_{ST} among the 20 collection sites.

Population	Montana[1] Mining Ditch	Decker[2]/ Sullivan	Unm.[3] Pottery House	Malad[4] River	Lower[5] White Springs	Billingsley[6] Creek	Fisher[7] Lake	Riley[8] Creek (upper)	Riley[9] Creek (lower)	Bickel[10] Springs (upper)	Bickel[11] Springs (lower)	Thousand[12] Springs	Sculpin[13] Springs	Sand[14] Springs	Blue[15] Hearts Springs	Box[16] Canyon (lower)	Box[17] Canyon (upper)	Blind[18] Canyon	Banbury[19] Springs
Decker/Sullivan[2]	0.01																		
Unm. Pottery House[3]	0.06	0.04																	
Malad River[4]	0.21	0.24	0.23																
Lower White Springs[5]	0.20	0.23	0.22	0.02															
Billingsley Creek[6]	0.26	0.27	0.27	0.17	0.15														
Fisher Lake[7]	0.25	0.27	0.27	0.20	0.17	0.01													
Riley Creek (upper)[8]	0.12	0.14	0.19	0.26	0.22	0.21	0.21												
Riley Creek (lower)[9]	0.26	0.30	0.29	0.11	0.12	0.24	0.28	0.28											
Bickel Springs (upper)[10]	0.23	0.25	0.25	0.23	0.19	0.12	0.14	0.12	0.26										
Bickel Springs (lower)[11]	0.29	0.32	0.31	0.11	0.13	0.26	0.29	0.30	<0.00	0.28									
Thousand Springs[12]	0.30	0.34	0.33	0.12	0.14	0.27	0.31	0.32	<0.00	0.30	<0.00								
Sculpin Springs[13]	0.30	0.33	0.33	0.12	0.15	0.28	0.32	0.31	<0.00	0.30	<0.00	<0.00							
Sand Springs[14]	0.30	0.33	0.33	0.13	0.15	0.29	0.32	0.32	0.01	0.30	<0.00	<0.00	<0.00						
Blue Hearts Springs[15]	0.35	0.38	0.40	0.18	0.21	0.39	0.41	0.37	0.09	0.37	0.10	0.09	0.06	0.07					
Box Canyon (lower)[16]	0.32	0.36	0.37	0.16	0.18	0.36	0.40	0.35	0.07	0.35	0.08	0.08	0.05	0.06	<0.00				
Box Canyon (upper)[17]	0.37	0.42	0.43	0.26	0.27	0.44	0.46	0.41	0.17	0.42	0.18	0.17	0.16	0.17	0.13	0.09			
Blind Canyon[18]	0.32	0.35	0.36	0.16	0.19	0.35	0.39	0.35	0.07	0.34	0.09	0.08	0.06	0.07	<0.00	<0.00	0.10		
Banbury Springs[19]	0.51	0.52	0.53	0.30	0.33	0.51	0.51	0.51	0.20	0.51	0.20	0.18	0.17	0.18	0.15	0.14	0.25	0.13	
BriggsCreek[20]	0.60	0.59	0.62	0.38	0.39	0.60	0.55	0.59	0.29	0.59	0.31	0.29	0.26	0.27	0.24	0.20	0.36	0.19	0.15

Table 3. Effective population size estimates from LDNE (with 95% CI) of varying sample sizes (50, 100, 150, 200, 250, 300) for the Fisher Lake population.

N	N_E	$N_E^{(95\%L)}$	$N_E^{(95\%U)}$
50	149.8	37.0	∞
100	329.9	91.9	∞
150	4119.4	207.5	∞
200	-1473.6	487.8	∞
250	1729.6	277.8	∞
300	2156.2	401.1	∞

Table 4. Tests for past bottlenecks in population size using two tests (Sign and Wilcoxon) under two models of microsatellite mutation (TPM and SMM). P-values <0.05 are considered significant. When p-values <0.05 for the Sign test, the number of loci with heterozygosity deficiency (D) is shown out of the total loci examined (12).

Population	Collection Site #	Sign Test TPM	Sign Test SMM	Wilcoxon Test (Deficiency) TPM	Wilcoxon Test (Excess) TPM	Wilcoxon Test (Deficiency) SMM	Wilcoxon Test (Excess) SMM
Montana Mining Ditch	1	0.57	0.31	0.52	0.52	0.31	0.72
Decker/Sullivan	2	0.30	0.12	0.31	0.72	0.12	0.90
Unm. Pottery House	3	0.32	0.13	0.28	0.74	0.08	0.94
Malad River	4	0.13	0.04 D8/12	0.21	0.82	0.01	0.99
Lower White Springs	5	0.08	0.00 D10/12	0.06	0.95	0.00	1.00
Billingsley Creek	6	0.47	0.24	0.72	0.31	0.28	0.75
Fisher Lake	7	0.02 D9/12	0.00 D11/12	0.02	0.99	0.00	1.00
Riley Creek (upper)	8	0.21	0.07	0.22	0.81	0.01	0.99
Riley Creek (lower)	9	0.07	0.00 D10/12	0.10	0.91	0.00	1.00
Bickel Springs (upper)	10	0.54	0.25	0.31	0.72	0.10	0.92
Bickel Springs (lower)	11	0.36	0.00 D10/12	0.34	0.69	0.00	1.00
Thousand Springs	12	0.16	0.00 D112/12	0.12	0.90	0.00	1.00
Sculpin Springs	13	0.17	0.00 D11/12	0.31	0.72	0.00	1.00
Sand Springs	14	0.07	0.00 D10/12	0.09	0.92	0.00	1.00
Blue Hearts Springs	15	0.08	0.08	0.26	0.77	0.05	0.96
Box Canyon (lower)	16	0.00 D10/12	0.00 D11/12	0.00	1.00	0.00	1.00
Box Canyon (upper)	17	0.20	0.02	0.10	0.91	0.01	0.99
Blind Canyon	18	0.02	0.00 D10/12	0.06	0.95	0.00	1.00
Banbury Springs	19	0.02	0.00 D10/12	0.00	1.00	0.00	1.00
Briggs Creek	20	0.15	0.14	0.14	0.88	0.07	0.95

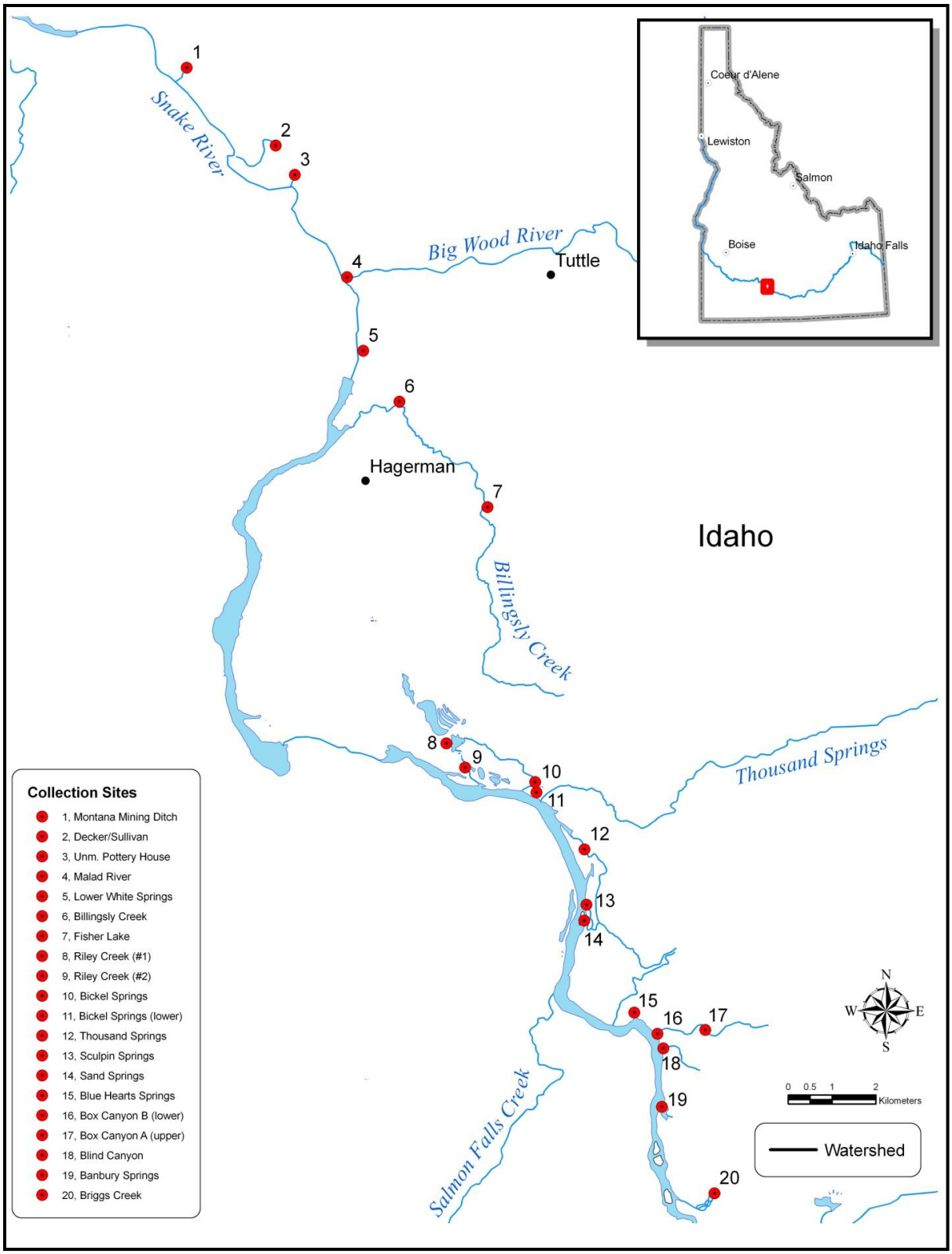

Figure 1. Locations of the 20 collections sites for Shoshone sculpin across their range in the Hagerman Valley, Idaho.

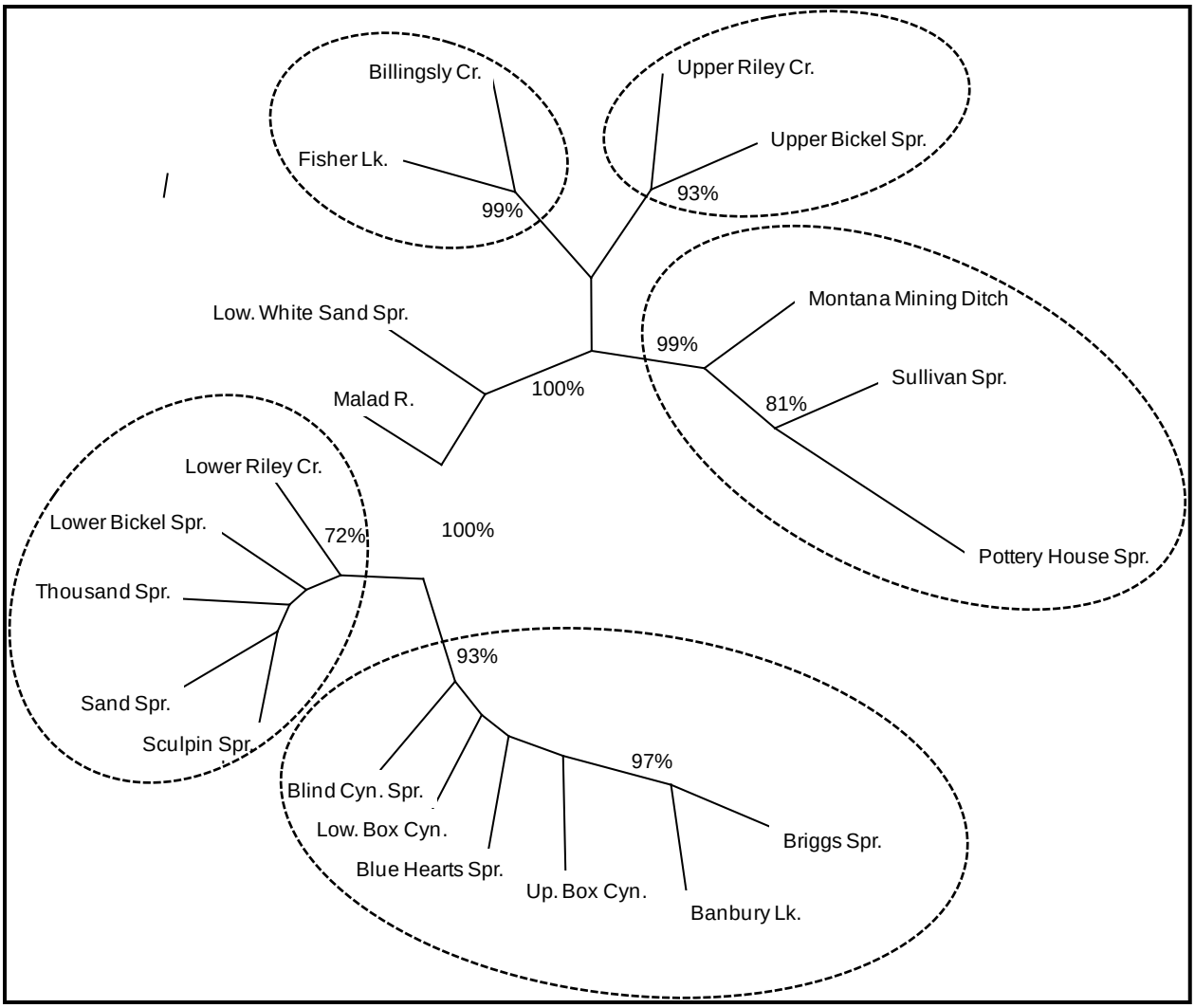

Figure 2. Dendrogram showing population relationships (Neighbor-Joining tree based on Cavalli-Sforza and Edwards [1967] genetic chord distances). Bootstrap values are reported as percentages of the total and were listed only if they exceeded 70%.

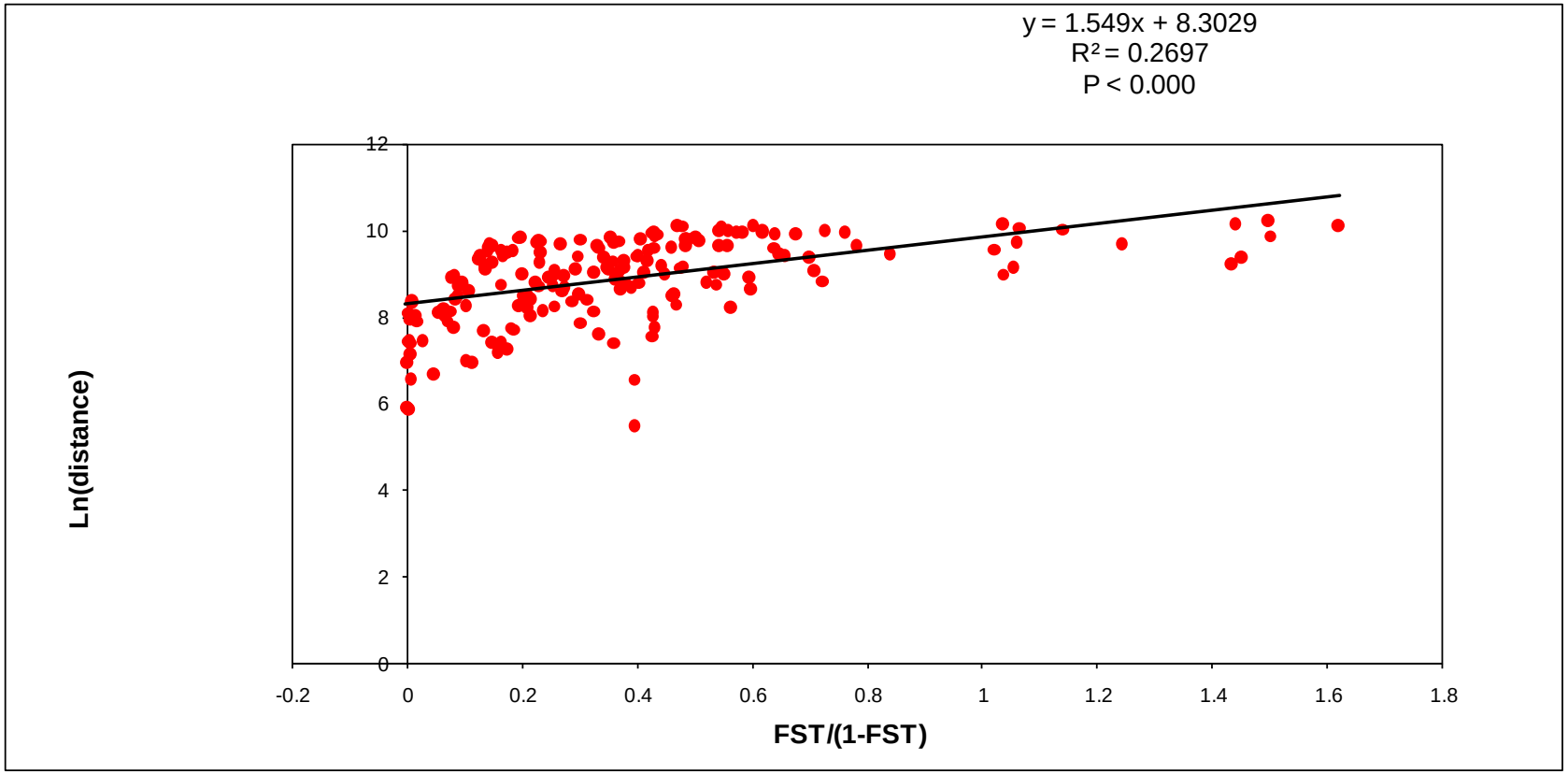

Figure 3. Scatter plot of pairwise genetic (FST/(1-FST)) versus geographic distance (Ln) of 20 Shoshone sculpin populations showing a significant pattern of isolation by distance.

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